hydrolysates of α^A and α^F chains are grossly indistinguishable[6] but also that α chains may be transferred by hybridization of hemoglobin F with other hemoglobins.

All hemoglobins were isolated from hemolysates by column chromatography[7] to remove minor components normally present. Zone F_{II},[7] the main component of normal full-term cord blood, was taken as hemoglobin F.

The reversible dissociation of hemoglobin F into molecular sub-units is similar but not identical to hemoglobin A[8,9] at both alkaline and acid *p*H. Thus hybridization[10,11] or recombination[10] with other hemoglobins might be expected. Initial hybridization of hemoglobin F_{II} ($\alpha_2^F\gamma_2^F$) and radioactive (C^{14}-labelled leucine) hemoglobin S ($\alpha_2^{A*}\beta_2^{S*}$) at *p*H 11.0 or 11.2 at 3° for 1 to 6 days showed less transfer of radioactivity to hemoglobin F than would have been expected from a hybridization of the type

$$2\,\alpha_2^{F=A}\gamma_2^F + 2\,\alpha_2^{A*}\beta_2^{S*} \rightarrow \alpha_2^{F=A}\gamma_2^F + \alpha_2^{A*}\gamma_2^F + \alpha_2^{A*}\beta_2^{S*} + \alpha_2^{F=A}\beta_2^{S*} \quad (1)$$

if α^F and α^A are identical. Nevertheless, hemoglobin F_{II} after chromatographic isolation was radioactive in the α chains only, as shown by investigation of the N-terminal peptides.[2,4,12] Likewise, when radioactive hemoglobin F_{II} and hemoglobin A were hybridized at *p*H 4.7 at 3° for 12 hr., transfer of radioactivity to hemoglobin A was 53% of that calculated from an equation like (1). These experiments do not determine the number of identical α chains in hemoglobin F. However, after approximately equal amounts of hemoglobin F_{II} and hemoglobin H (β_4^A)[13] had been hybridized at *p*H 4.7 at 3° for 12 hr., chromatography separated three hemoglobins, an "H-like" hemoglobin, "F_{II}", and "A." From the ratio of F_{II} to A which was very close to 1:2, it was calculated that hybridization according to the equation

$$3\alpha_2^{F=A}\gamma_2^F + 3\beta_4^A \rightarrow 2(2\beta_2^A + \gamma_2^F) + \alpha_2^{F=A}\gamma_2^F + 2\alpha_2^{F=A}\beta_2^A \quad (2)$$

was essentially complete (97%): thus, two identical α chains are present in hemoglobin F. The newly formed hemoglobin A was characterized by its chromatographic and starch electrophoretic behavior. The nature of the "H-like" hemoglobin from the hybridization is unknown at present. At *p*H 11.2, the hybridization of F_{II} and H was about 20% in 12 hr.

From the incompleteness of hybridization under certain conditions, the process clearly is a complex one. As more information becomes available, it should give an insight into the apparently different forces between the chains of the various hemoglobins.

The conclusion that the α^F and α^A chains are identical strongly suggests a related genetic control of hemoglobins A and F and is an extension of the idea derived from the structure of hemoglobin H that the α and β chains of adult hemoglobin are under separate genetic control.[13,14] It is clearly apparent that these ideas would be substantiated by the detection of an abnormal fetal hemoglobin in a newborn child who, in adult life, will produce an abnormal hemoglobin that has an aberration in the α chain.

This investigation was supported in part by grants H-2558 and H-3394 from the National Institutes of Health, United States Public Health Service.

(6) J. A. Hunt (*Nature*, **183**, 1373 (1959)) likewise concludes that α^A and α^F chains are identical on the basis of a somewhat more extensive study of "fingerprint" data which included examination of the chymotryptic digest of the insoluble tryptic residue. We wish to thank Mr. Hunt for sending us a copy of his manuscript prior to publication.

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MICROBIOLOGICAL HYDROXYLATION OF 12a-DEOXYTETRACYCLINE

Sir:

We wish to report on the ability of *Curvularia lunata* (NRRL 2380) to form tetracycline by introduction of the 12a-hydroxyl group into the compound 12a-deoxytetracycline.[1]

Curvularia lunata was grown for 40 hours in shaker flasks containing a medium consisting of 2% corn meal, 1% N-Z Amine B, 0.2% Difco yeast extract, 0.1% K_2HPO_4 and 0.5% $CaCO_3$. To 1.4 liters of culture filtrate was added 1.5 g. of 12a-deoxytetracycline as a 20 mg./ml. aqueous solution (*p*H to 1.5 with HCl). The final *p*H of the reaction mixture was 6.0. The reaction mixture was incubated at 28° for 24 hr. At this time, spectrophotometric assay indicated that 89% of the 12a-deoxytetracycline had been consumed. Microbiological assay of the harvest sample suggested a 23% yield of tetracycline. For isolation purposes the *p*H of the reaction mixture was raised to 8.5. It was then extracted three times with half volume portions of 1-butanol. The butanol extracts were combined, adjusted to *p*H 2.6 and concentrated to about 20 ml. The precipitate obtained by adding 200 ml. of petroleum ether was chromatographed on a Celite 545[2] column employing the solvent system ethyl acetate:1-butanol:H_2O (68:12:20) adjusted to *p*H 2.1 with HCl. Ultraviolet absorption of the effluent was continuously measured at 270 mμ and fractions representative of three peaks were collected. The crystalline product obtained from fraction three proved identical by ultraviolet and infrared ab-

(1) Details for the preparation of 12a-deoxytetracycline are to be published by A. Green and J. H. Boothe. The suggestion concerning the possibility of microbiological 12a-hydroxylation was made by A. Green.

(2) A grade of diatomaceous earth produced by the Johns-Manville Co.

sorption spectra, and by bioassay and paper chromatography with an authentic sample of tetracycline.

The active principles excreted by *Curvularia lunata* were non-dialyzable and were completely inactivated by heating in aqueous solution at 100° for 5 minutes. These observations suggest that the catalysts are enzymes. The *p*H optimum for tetracycline production and for 12a-deoxytetracycline utilization was about 4.6. Warburg studies revealed that oxygen was required and that it was the only gas involved in the reaction. Approximately 1 atom of oxygen was utilized per mole 12a-deoxytetracycline consumed. Since *Curvularia lunata*, preparations have no marked effect on tetracycline it is concluded that the products of the reaction are derived from 12a-deoxytetracycline and are not tetracycline degradation products. To account for the stoichiometry observed, the other reaction products must average 1 atom of additional oxygen per molecule.

Efforts to obtain crystalline material from fractions 1 and 2 have been unsuccessful. Additional column chromatography of fractions 1 and 2 yielded more cleanly resolved preparations, each of which by paper chromatography still contained more than one substance. The best preparation from fraction 1 showed ultraviolet absorption maxima in 0.1 *N* HCl characteristic of anhydro derivatives. Fraction 2 is believed to contain an 11a-hydroxylated product based on a consideration of the spectra obtained in 0.1*N* HCl and 0.1*M* sodium borate, and the fact that refluxing with methanolic HCl failed to yield an anhydro product.

While other cultures, *e.g.*, *Curvularia pallescens* and *Botrytis cinerea*, were also able to 12a-hydroxylate 12a-deoxytetracycline, several strains of *Streptomyces aureofaciens* were not.

Grateful acknowledgment is extended to A. Green and J. H. Boothe for supplies of 12a-deoxytetracycline, to A. C. Dornbush for microbiological assays, to J. H. Martin for paper chromatographic assays, and to W. Fulmor for infrared analyses.

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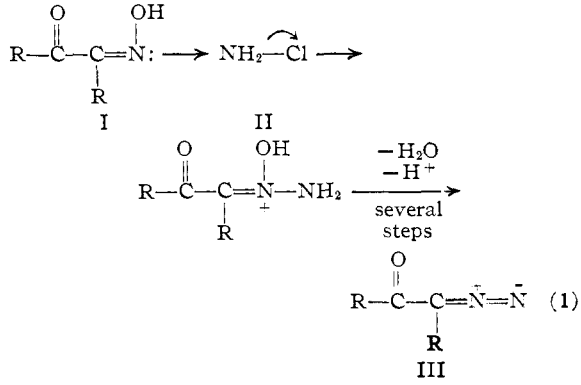
THE FORSTER REACTION AND DIAZOALKANE SYNTHESIS

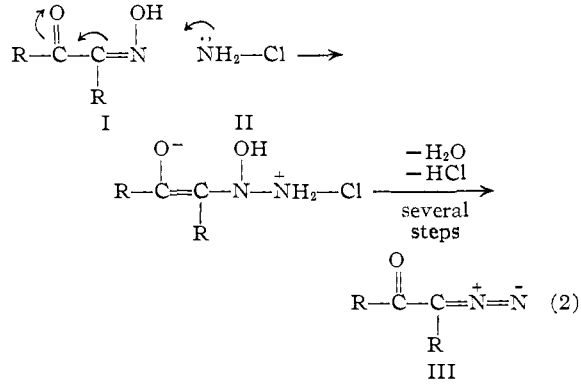
Sir:

The reaction of α-oximinoketones (I) with chloramine (II) to give diazoketones (III) was first reported by Forster in 1915.[1] As a technique for the preparation of cyclic diazoketones, this reaction has found several recent applications.[2,3,4] We have speculated about the mechanism of this transformation, and consider a sequence of steps initiated by a nucleophilic displacement at the chloramine nitrogen, as shown in equation (1), to be most plausible. The first step finds close analogy in the well-known Raschig



synthesis of hydrazine from chloramine and ammonia,[5] and more distant analogy in the Kocheshkov amine synthesis.[6] *A key feature of the process represented in (1) is the irrelevance of the carbonyl function.*

Alternate mechanisms, whose first step would be reminiscent of a Michael addition, also may be imagined for the Forster reaction. This type of process, outlined in equation (2), assigns a vital role to the carbonyl function.[7]



We have sought an experimental distinction between these two mechanisms, and wish to report results which support the nucleophilic displacement process (1) and provide a new route to diazoalkanes which may be of general interest.

Fluorenone oxime (0.50 g.) was suspended in 15 *N* ammonium hydroxide (25 ml.) and a 5.25% solution of sodium hypochlorite (100 ml.) was added at 0° over a one-hour period. The mixture was extracted with petroleum ether several times over a four-hour period. These extracts yielded diazofluorene (0.19 g.), identified by infrared comparison with an authentic sample. Unreacted oxime (0.25 g.) was recovered from the aqueous layer.

Similar experiments were carried out with the oximes of benzophenone, acetophenone and

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(5) For an interesting review of chloramine chemistry see E. Colton and M. M. Jones, *J. Chem. Ed.*, **32**, 485 (1955).

(6) For a leading reference see P. L. Pauson, *Quart. Rev.*, **9**, 413 (1955).

(7) The authors are grateful to Professors Gilbert Stork and Ronald Breslow for a stimulating discussion during which this possibility came to light.